

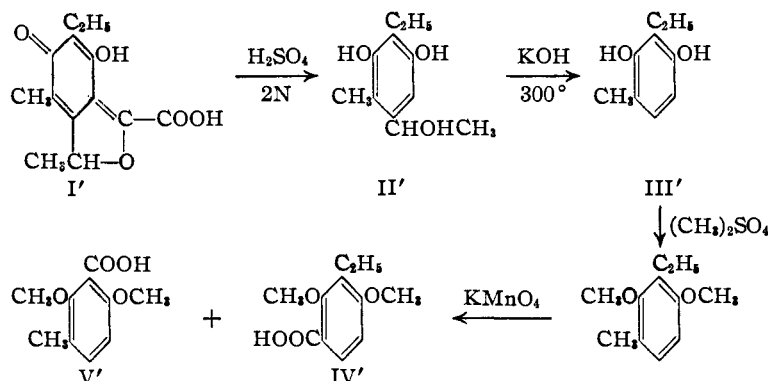
[CONTRIBUTION FROM THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Mold Metabolites. III. The Structure of Citrinin

BY DONALD J. CRAM¹

Citrinin was first isolated as a pure compound by Hetherington and Raistrick² from the culture liquors of *Penicillium citrinum*. Since that time this pigment has been isolated from numerous sources,^{3,4,5,6,7} and has been found to possess marked antibiotic properties.^{7,8,9}

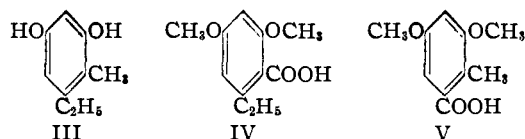
An investigation of the structure of citrinin (I) (C₁₃H₁₄O₆) was undertaken by Hetherington and Raistrick² who degraded the substance with acid to II (C₁₁H₁₆O₃), formic acid and carbon dioxide. Compound II when fused with alkali produced III (C₉H₁₂O₂), which in turn was methylated and oxidized to two carboxylic acids IV (C₁₁H₁₄O₄) and V (C₁₀H₁₂O₄). Coyne, Raistrick and Robinson¹⁰ ascribed a quinoidal structure to citrinin and interpreted the above reactions in the following manner. The structures that these workers assigned to IV and V rested on rather dubious color reactions. Sprenger and Ruoff¹¹ synthesized III',



IV' and V' and showed that the properties of these substances were different from those of III, IV and V, respectively. This work made structure I' for citrinin untenable, as well as all the structures assigned to the degradation products and derivatives by Coyne, *et al.*¹⁰ Gore, *et al.*,⁸ found that citrinin coupled with one mole of diazotized 2,5-dichloroaniline and that II coupled with two moles of diazotized aniline. These facts led the above investigators to discard I' for the structure of citrinin.

- (1) American Chemical Society Postdoctoral Fellowship, 1947-1948.
- (2) Hetherington and Raistrick, *Trans. Roy. Soc. (London)*, **B220**, 269 (1931).
- (3) Ewart, *Ann. Bot.*, **47**, 913 (1933).
- (4) Raistrick and Smith, *Biochem. J.*, **29**, 606 (1935).
- (5) Pratt, *et al.*, *Science*, **99**, 351 (1944).
- (6) Gore, Panse and Venkataraman, *Nature*, **157**, 333 (1945).
- (7) Pollock, *ibid.*, **180**, 331 (1946).
- (8) Raistrick and Smith, *Chem. and Ind.*, **60**, 829 (1931).
- (9) Tauber, Laufer and Goll, *THIS JOURNAL*, **64**, 2228 (1942).
- (10) Coyne, Raistrick and Robinson, *Trans. Roy. Soc. (London)*, **B220**, 297 (1931).
- (11) Sprenger and Ruoff, *J. Org. Chem.*, **11**, 189 (1946).

In the present investigation the compound 4-methyl-5-ethylresorcinol was synthesized by two different methods and a comparison of this substance with III (prepared from citrinin) showed that the two samples were identical. Two acids, 2-ethyl-4,6-dimethoxybenzoic acid and 2-methyl-3,5-dimethoxybenzoic acid, have also been synthesized, and their properties correspond to those reported for IV and V, respectively.



Formylation of 5-ethylresorcinol¹² with zinc cyanide and hydrogen chloride gas produced 2-ethyl-4,6-dihydroxybenzaldehyde.¹³ The two phenolic groups were methylated to give 2-ethyl-4,6-dimethoxybenzaldehyde, which in turn was oxidized to 2-ethyl-4,6-dimethoxybenzoic acid, m. p. 99-100° (Hetherington, *et al.*,² reported a melting point of 98-99° for IV).

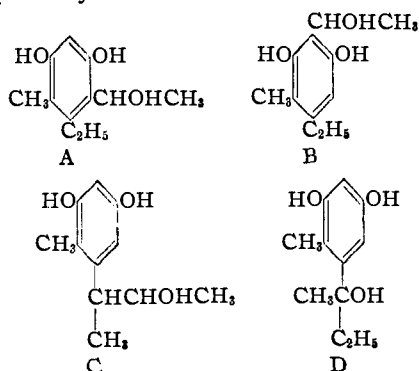
Reduction of 2-ethyl-4,6-dimethoxybenzaldehyde with hydrazine and alkali¹⁴ followed by demethylation of the two methoxyl groups produced 4-methyl-5-ethylresorcinol, m. p. 67-69°. A mixed melting point of this compound with III¹⁵ prepared from citrinin gave no depression. The bis-(*p*-nitrobenzoate) of 4-methyl-5-ethylresorcinol was prepared, and a mixed melting point with the bis-(*p*-nitrobenzoate) of III gave no depression.

Methylation of the hydroxyl groups of 2-methyl-3,5-dihydroxybenzoic acid (this compound was prepared by the method of Jacobsen and Wierss¹⁶) produced 2-methyl-3,5-dimethoxybenzoic acid, m. p. 157-158° (Hetherington, *et al.*,² reported a melting point of 142-146° for V). The discrepancy between the two melting points suggests that the material derived from citrinin was impure, which fact would explain its four-degree melting range.

- (12) This compound was prepared by the procedure of Asahina and Ihara, *J. Pharm. Soc. Japan*, **48**, 28 (1928).
- (13) This substance had been previously prepared by Geissman and Tulagin (unpublished work), m. p. 199-200°.
- (14) A procedure similar to that developed by Huang-Minlon [*THIS JOURNAL*, **68**, 2487 (1946)] was employed.
- (15) Hetherington, *et al.*,² reported a melting point of 97-99° for this substance. After the sample had stood for some time the melting point changed to 65-70°. Gore, *et al.*,⁸ reported a melting point of 68-69° for III and stated that the molecule contained water of crystallization. Sprenger, *et al.*,¹¹ reported a melting point of 67-70°.
- (16) Jacobsen and Wierss, *Ber.*, **16**, 1960 (1883).

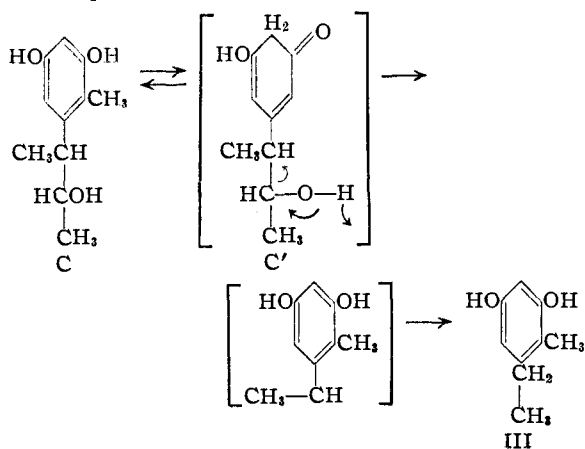
Conversion of the above acid to the amide followed by treatment with methylmagnesium bromide gave 2-methyl-3,5-dimethoxyacetophone. Reduction of the ketone with hydrazine and alkali¹⁴ followed by demethylation of the two methoxyl groups produced 4-methyl-5-ethylresorcinol, m. p. 67–69°. Admixture of this sample with III or the sample prepared from 5-ethylresorcinol produced no depression.

Terminal-methyl number determinations were made on citrinin (2.68 and 2.76) and on II (2.57 and 2.51). These data indicate that both compounds have a minimum of three terminal methyl groups, and these facts coupled with the conversion of II to III by fusion with alkali leaves four possible structures for II, namely, A, B, C and D. Gore, *et al.*,⁸ found that two molecules of 2-methoxy-4-nitrobenzenediazonium chloride coupled in an apparently normal manner with II, but that an



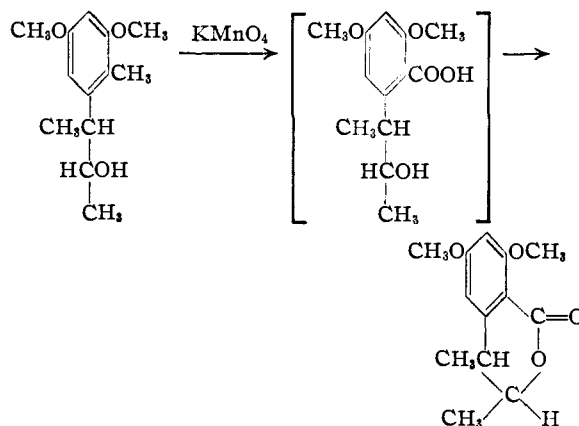
anomalous product was obtained when benzenediazonium chloride was employed. In the latter case an analysis of the product was obtained that corresponds to a bis-diazo compound minus one mole of water. It is difficult to formulate these reactions on the basis of structure A or B. Both C and D have two positions available for coupling with diazonium chlorides. Of these two formulas, C can accommodate the reactions that Hetherington, *et al.*,² found characteristic of II, whereas D is completely untenable.

Compound C would form a dimethyl ether, an



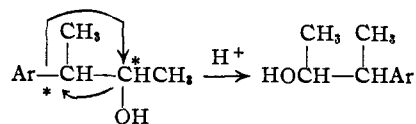
acetate of the dimethyl ether, and a di- and a triacetate. Fusion of C with alkali would lead to a reverse aldol condensation in the manner shown below. The two hydroxyl groups in the nucleus would aid in the cleavage by virtue of the fact that the tautomer C' is a vinylog of a β -hydroxy ketone.

The oxidation of the dimethyl ether of C to a lactone of molecular formula $C_{13}H_{16}O_4$ (Hetherington, *et al.*,² reported that the dimethyl ether of II could be so oxidized but in very poor yield) can be formulated as the conversion of the methyl group to a carboxyl group followed by lactone formation.



Oxidation of the secondary alcohol in the side chain to a ketone is probably the main reaction and explains the low yield of the lactone.

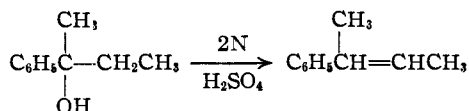
Formula C contains two asymmetric carbon atoms and therefore can account for the optical activity of II. Hetherington, *et al.*,² reported that the racemate of II was obtained as a product of both acid hydrolysis of citrinin as well as from an acetoxy determination run on the triacetate of II. The racemization of II has also been realized in the present investigation by the treatment of II with boiling 6 *N* sulfuric acid for four hours. Treatment of II with a boiling 2 *N* sodium hydroxide solution did not affect the properties of II. From these facts it is safe to assume that the racemization is acid catalyzed. Since the racemization of C cannot be explained on the basis of simple racemization of the carbon atom bearing the hydroxyl group (the adjacent carbon atom is also asymmetric), it probably takes place by means of a Wagner-Meerwein rearrangement involving the aryl group as a migrating species. The product



would be structurally identical to the starting material, but the configuration of both carbon atoms would be altered. The conditions used in the racemization are those commonly found in Wagner-Meerwein rearrangements, and work is under way in this Laboratory to attempt to estab-

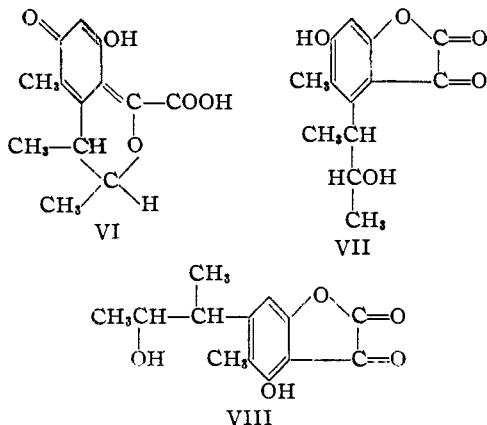
lish the above formulation of the racemization through the use of model compounds, and to establish the steric relationships involved. The synthesis of C has also been undertaken.

Structure D is entirely incompatible with the chemistry of II. Tertiary alcohols racemize so quickly in hydrolytic solvents that a successful resolution of a tertiary alcohol has never been accomplished. Since II is produced by the treatment of citrinin with boiling 2 *N* sulfuric acid it seems improbable that a molecule of structure D could maintain its optical integrity. In the present investigation methylethylphenylcarbinol was prepared as a model for D. Treatment of this substance with boiling 2 *N* sulfuric acid resulted in complete dehydration of the substance to 2-phenylbutene-2. The production of III from II is also impossible to formulate on the basis of structure D. The formation of the triacetate of



II through the use of sodium acetate and acetic anhydride cannot be reconciled with the fact that if D were II, the reaction would involve an acetylation of a tertiary alcohol, and such a reaction could not be accomplished in the present investigation with the use of phenylmethylethylcarbinol as a model substance.

On the basis of the evidence now at hand citrinin can be formulated as having one of the three structures VI,¹⁷ VII or VIII. The results of a spectral study of citrinin and model compounds for structures VI, VII and VIII will be published shortly.



Experimental

2-Ethyl-4,6-dimethoxybenzaldehyde.—From 3,5-dimethoxybenzoic acid, an 80% yield of 3,5-dimethoxybenzamide was obtained by the method of Suter and Weston,¹⁸ m. p. 144.5° (lit.¹⁸ 146°). Treatment of this compound

(17) Gore, Panse and Venkataraman [THIS JOURNAL, 70, 2287 (1948)] suggested this structure for citrinin. The formula is a modification of the structure advanced by Coyne, *et al.*,¹⁰ and it accommodates all the evidence now available on the constitution of citrinin.

(18) Suter and Weston, THIS JOURNAL, 61, 232-236 (1939).

with methylmagnesium bromide by the procedure used by Suter and Weston¹⁸ to prepare similar ketones produced a 63% yield of 3,5-dimethoxyacetophenone, m. p. 45° (lit.¹⁹ 42-43°). Reduction of the ketonic group and demethylation of the two methoxyl groups was accomplished by the procedures of Asahina and Ihara.¹² The yield of 3,5-dihydroxyethylbenzene amounted to 52%, m. p. 92-93° (lit.¹² 93°). Formylation of the substance with zinc cyanide and hydrogen chloride gas produced a 62% yield of 2-ethyl-4,6-dihydroxybenzaldehyde,¹³ m. p. 198-199°. This aldehyde (250 mg.), dissolved in 5 cc. of boiling methanol, was alternately treated with 0.5-cc. portions of dimethyl sulfate and 0.5-cc. portions of a 40% potassium hydroxide solution. After a total of 3 cc. of dimethyl sulfate had been added over a period of ten minutes the mixture was cooled, made basic, flooded with water and extracted with ether. This extract was dried, evaporated and the product recrystallized from ethanol (white needles) to give 190 mg. of 2-ethyl-4,6-dimethoxybenzaldehyde, m. p. 50-51°.

Anal. Calcd. for C₁₁H₁₄O₃: C, 68.03; H, 7.27. Found: C, 68.19; H, 7.23.

2-Ethyl-4,6-dimethoxybenzoic Acid.—A mixture of 100 mg. of 2-ethyl-4,6-dimethoxybenzaldehyde, 2 ml. of 0.2 *N* potassium permanganate solution and 1 ml. of a 0.001 *N* sodium hydroxide solution was boiled for ten minutes, filtered and acidified. The product that separated was recrystallized from water to give 56 mg. of white needles, m. p. 99-100° (Hetherington, *et al.*,² reported 98-99° for IV).

Anal. Calcd. for C₁₁H₁₄O₄: C, 62.84; H, 6.71. Found: C, 62.76; H, 6.71.

4-Methyl-5-ethylresorcinol.—A mixture of 150 mg. of 2-ethyl-4,6-dimethoxybenzaldehyde, 0.25 g. of potassium hydroxide, 0.2 ml. of hydrazine hydrate and 2 ml. of ethylene glycol was refluxed for one hour and then heated to 185-190° for four hours (during this period the apparatus was set for distillation). At the end of this time the distillate was mixed with the material in the distilling flask, the mixture was flooded with water, extracted with ether, and the ether layer was washed with water, dried and evaporated to an oil. This oil was refluxed for four hours with 4 ml. of 48% hydrobromic acid. The solution was then cooled, diluted with water, extracted with ether, and the ether layer was washed with water, dried, treated with charcoal, filtered and evaporated to an oil. This oil was distilled at 1 mm. and crystallized twice from water, weight 60 mg., m. p. 67-69°. This material gave a transient blue color in aqueous solution to which a trace of ferric chloride was added. A mixed melting point of the substance (4-methyl-5-ethylresorcinol) with III (m. p. 67-69° from water) prepared from citrinin by the method of Hetherington, *et al.*,² gave no depression.

The bis- (*p*-nitrobenzoate) of 4-methyl-5-ethylresorcinol (and of III) was prepared by dissolving a small amount of the phenol in 1 ml. of pyridine, adding an equal amount of *p*-nitrobenzoyl chloride, heating the mixture to boiling, cooling and flooding the solution with water. This mixture was then extracted with ether, the ether layer was extracted with sodium carbonate, washed with water, dried, evaporated to a small volume and cooled. The material that crystallized melted at 205-206° in each case, and further recrystallization did not change the melting point. A mixed melting point of the two preparations gave no depression.

Anal. Calcd. for C₂₃H₁₈O₈N₂: C, 61.33; H, 4.03. Found: C, 61.50; H, 4.03.

2-Methyl-3,5-dimethoxybenzoic Acid.—Sulfonation of *o*-toluic acid followed by fusion of the product with alkali by the method of Jacobsen and Wiers¹⁶ gave a 5% yield of 2-methyl-3,5-dihydroxybenzoic acid, m. p. 243-244° (lit.¹⁶ 245°). Attempts to improve this yield failed (Jacobsen and Wiers¹⁶ did not report their yield).

Methylation of the above acid (4.5 g.) with dimethyl sulfate and alkali in a solution of boiling methanol in the usual

(19) Mautner, *J. prakt. Chem.*, 118, 275 (1927).

manner gave 4.6 g. of 2-methyl-3,5-dimethoxybenzoic acid, m. p. 157–158° (needles from ethanol). Hetherington, *et al.*,² reported a melting point of 142–146° for V.

Anal. Calcd. for $C_{10}H_{14}O_4$: C, 61.21; H, 6.16; neut. eq., 196. Found: C, 61.35; H, 6.55; neut. eq., 195.

2-Methyl-3,5-dimethoxybenzamide.—A mixture of 3.8 g. of 2-methyl-3,5-dimethoxybenzoic acid and 4.0 g. of phosphorus pentachloride was allowed to stand for one hour in a loosely stoppered flask. The resulting oil was heated to 100° for one-half hour, dissolved in benzene and stirred into a mixture of cracked ice and concentrated ammonium hydroxide. The precipitate that separated was collected and recrystallized from benzene to give white needles (3.3 g.), m. p. 160–161°. Further recrystallization did not alter the melting point.

Anal. Calcd. for $C_{10}H_{13}O_3N$: C, 61.52; H, 6.71. Found: C, 61.73; H, 7.04.

2-Methyl-3,5-dimethoxyacetophenone.—To a Grignard reagent prepared from 1.86 g. of magnesium and 11 g. of methyl iodide in 50 ml. of dry ether was added 3.0 g. of 2-methyl-3,5-dimethoxybenzamide. The mixture was stirred in an atmosphere of nitrogen for forty hours, cooled and poured into a mixture of ice and 20% sulfuric acid. The resulting solution was boiled for one-half hour, cooled, extracted with ether, and the ether layer was washed successively with dilute solutions of sodium bisulfite, sodium bicarbonate and hydrochloric acid. The organic layer was then dried and evaporated to an oil, which was distilled at 1 mm. The distillate crystallized and was recrystallized from ether to give white prisms (2.3 g.), m. p. 46–47°.

Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.03; H, 7.27. Found: C, 68.28; H, 7.28.

4-Methyl-5-ethylresorcinol.—A mixture of 500 mg. of 2-methyl-3,5-dimethoxyacetophenone, 0.4 g. of potassium hydroxide, 0.5 ml. of hydrazine hydrate and 3 ml. of triethylene glycol was heated for one hour under reflux at 140°, and the temperature was raised to 185° and held there for four hours, the apparatus being set for downward distillation. At the end of this time the distillate and the material in the distilling flask were combined, flooded with water, extracted with ether, and the ether extract was washed with water, dried and evaporated to an oil. This oil was heated at reflux with 5 ml. of 48% hydrobromic acid for four hours, and the resulting solution was cooled, diluted with water, extracted with ether, and the ether layer was washed with water, dried and evaporated to an oil. Crystallization and recrystallization of the substance from water produced 200 mg. of 4-methyl-5-ethylresorcinol, m. p. 67–69°, not depressed by admixture with III from citrinin. The bis-(*p*-nitrobenzoate) of the substance was

prepared, m. p. 204–205°, not depressed by admixture with the bis-(*p*-nitrobenzoate) of III.

Racemization of II Obtained from Citrinin.—Citrinin (1.0 g.) was treated with acid by the procedure of Hetherington, *et al.*,³ and a total of 420 mg. of II was obtained (m. p. 128–130°) along with 100 mg. of the racemate of II (m. p. 168–170°). A solution of 100 mg. of II in 100 cc. of 6 *N* sulfuric acid was boiled under reflux for four hours, cooled, and extracted with ether. The ether solution was dried, evaporated, and the resulting oil was sublimed at 1 mm. pressure. The product was then crystallized and recrystallized from an ethyl acetate–petroleum ether mixture to give 40 mg. of the racemate of II, m. p. 167–168°. A mixed melting point with the racemate of II obtained directly from citrinin produced no depression.

Dehydration of Methyleneethylphenylcarbinol.—Methyleneethylphenylcarbinol was prepared according to the method of Klages,²⁰ b. p. 104–105° (18 mm.), n_D^{20} 1.5150. A mixture of 25 g. of the substance and 200 cc. of 4 *N* sulfuric acid solution was refluxed for four hours, cooled, extracted with ether, and the ether layer was washed with water, dried and evaporated. The product was distilled and the 2-phenylbutene-2²⁰ was collected at 83–84° (18 mm.), n_D^{20} 1.5282, weight 20.3 g.

Acknowledgment.—The author takes pleasure in expressing appreciation to T. A. Geissman for many suggestions, to Merck & Co., Inc., for a sample of citrinin, and to Welton Burney for the analyses reported in this paper.

Summary

The syntheses of three degradation product of citrinin have been reported, 2-ethyl-4,6-dimethoxybenzoic acid, 2-methyl-3,5-dimethoxybenzoic acid and 4-methyl-5-ethylresorcinol, the last compound by two different methods. Comparison of 4-methyl-5-ethylresorcinol with an authentic sample of degradation product prepared from citrinin proved the compounds to be identical. The properties of II (the initial degradation product of citrinin) have been shown to be compatible only with the structure 4-methyl-5-(1-methyl-2-hydroxypropyl)-resorcinol. Three possible structures for citrinin have been considered.

(20) Klages, *Ber.*, **35**, 3507 (1902).

LOS ANGELES, CALIFORNIA

RECEIVED JUNE 9, 1948

NOTES

β -Tetralone

By HOMER ADKINS, A. G. ROSSOW AND JAMES E. CARNAHAN

Recent interest^{1,2} in β -tetralone may make the process, used in this Laboratory³ for its preparation, of some value to others.

β -Naphthol, 500 g., in 250 ml. of dry alcohol was hydrogenated over 50 g. of copper chromium oxide at 200° under 1500 to 2500 p.s.i. until 2 moles of hydrogen per mole of

naphthol had been taken up during about five hours.⁴ The catalyst was separated by centrifuging and the alcohol by distillation. The residue was taken up in 600 ml. of benzene and washed with ten 200-ml. portions of a 10% sodium hydroxide solution and finally with 200 ml. of water. After distillation of the benzene and 15–20 ml. of intermediate fraction, there was obtained 409 g. (80%) of product, n_D^{20} 1.5632–1.5637, distilling 140–141° (13 mm.).

Dauben, McKusick and Mueller⁵ have shown that the product so prepared and isolated may contain a few per cent. of the isomeric 5,6,7,8-tetrahydro-2-naphthol.

(1) Stork and Foreman, *THIS JOURNAL*, **68**, 2172 (1946).

(2) Birch, *J. Chem. Soc.*, 430 (1944).

(3) A. G. Rossow, Ph.D. Thesis, University of Wisconsin, 1942.

(4) Adkins and Reid, *THIS JOURNAL*, **63**, 741 (1941).

(5) Dauben, McKusick and Mueller, *ibid.*, **70**, 4179 (1948).